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GJ Kant and RA Bauman

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Department of Medical Neurosciences
Division of Neuropsychiatry
Walter Reed Army Institute of Research
Washington DC, 20307-5100

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13. ABSTRACT (Maximum 200 words)

Stress is thought to affect the quantity and preference for food in humans. In this experiment, rats were allowed to leverpress for two types of food pellets of equivalent caloric value but different sucrose content during a 16 day baseline and then a 14 day period of stress. The chronic stressor was signalled around-the-clock intermittent footshock. One group of rats had control over stressor termination while a second group was yoked to the escape performance of the rats in the first group. We have previously reported that rats in this model of chronic stress tolerate the paradigm well, continuing to gain weight, eat, drink water, and groom and escape more than 99% of the trials presented. During the prestress baseline period, the sweeter pellet was preferred by most rats but differences in preference among rats and in preference at different times of day were observed. The preference for the high sucrose pellet was most marked in the hours preceding lights off. Overall, no changes in food preference were seen as a function of stress condition during the 14 day stress period.

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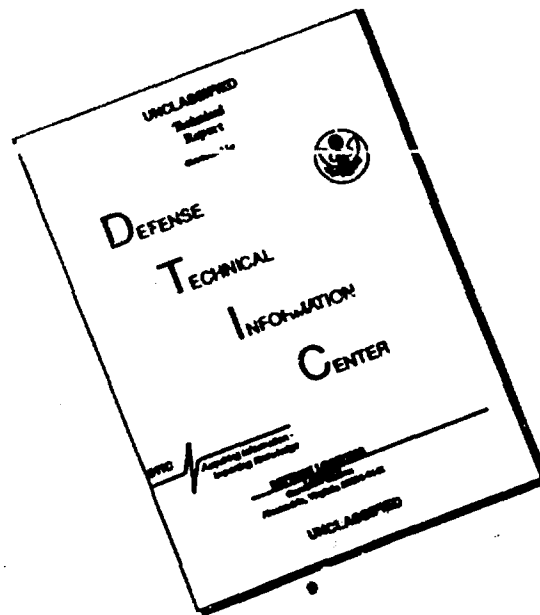
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Effects of Chronic Stress and Time of Day on Preference for Sucrose

G. JEAN KANT¹ AND RICHARD A. BAUMAN

Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100

KANT, G. J. AND R. A. BAUMAN. *Effects of chronic stress and time of day on preference for sucrose*. *PHYSIOL BEHAV* 54(3) 499-502, 1993.—Rats were allowed to lever press for two types of food pellets of equivalent caloric value and total carbohydrate content. One type of food pellet provided more of the calories as sucrose. During a 16-day prestress period, lever presses for 12 rats were recorded hourly. Following the baseline period, four rats (stressed group) were shaped to pull a ceiling chain to avoid or escape signalled foot shock presented intermittently around-the-clock. Four additional rats (yoked group) were each paired to one of the chain-pulling rats such that the rat trained to pull the ceiling chain controlled stressor termination for both rats. A third group of four rats served as the control group and received no shock. We have previously reported that rats in this model of chronic stress tolerate the paradigm well, continuing to gain weight, eat, drink water, and groom and escape more than 99% of the trials presented. During the baseline period, the sweeter pellet was preferred by most rats, but differences in preference among rats and in preference at different times of day were observed. The preference for the high-sucrose pellet was most marked in the hours preceding lights off. Overall, no changes in food preference were seen as a function of stress condition during the 14-day stress period, although one rat in the yoked group increased preference for the sweeter pellet during stress and returned to prestress food preferences when stress was terminated.

Stress Eating Circadian rhythm Sucrose Carbohydrate

STRESS is thought to be a contributing factor to many human diseases, including cardiovascular and gastrointestinal dysfunction, infectious disease, and mental illness (1,4,6,7,11,20,22,23). Our laboratory has been engaged in a systematic study of the effects of chronic stress on physiology and behavior using a rodent paradigm of stress that models some of the features of human stress. The model attempts to provide continuous moderate stress and sleep disruption, and allows for the experimental assessment of the role of available coping mechanisms in determining the physiological and behavioral consequences of stress exposure (2,3,5,10,13-15). In this paradigm, rats must avoid or escape signalled foot shock at approximately 5-min intervals around-the-clock. Other rats are yoked to the rats with control over shock termination such that pairs of stressed and yoked rats are exposed to the same stressor duration. We have reported that rats in this chronic stress paradigm continue to eat, gain weight, groom, and generally appear to tolerate the paradigm well. The rats with control over stressor termination quickly learn to pull a ceiling chain to avoid or escape foot shock and maintain this performance for weeks, without apparent difficulty, generally failing to avoid or escape less than three trials per day. However, decreased food consumption, elevated plasma levels of the stress-responsive hormones, corticosterone and prolactin, and disruptions in circadian rhythms of temperature and meal patterning demonstrate that rats in this paradigm are significantly stressed

and suggest that this paradigm may be a useful model of some types of chronic stress in humans.

One behavior that appears to be affected by stress is eating. In animal studies of chronic stress, decreased weight gain and food consumption have been frequently reported (9,15-17,19,24). In rats, stress has been reported to increase finickiness such that consumption of quinine-adulterated food is decreased (9). In humans, anecdotal reports would suggest that stress not only affects the amount of food eaten, but also the type of food preferred. That is, stress is popularly believed to affect consumption of sweets or other junk food.

In the present study, we hypothesized that chronic stress would increase preference for the sweeter of two food choices when rats in the above paradigm were given access to different choices available by lever press. We selected two pellets of markedly different sucrose content (5% vs. 53%) and conducted preliminary studies to establish the number of pellets eaten when only one of the two pellet types was available. We then conducted the main experiment where rats were allowed a choice between pressing two levers that each delivered one of the two pellets.

METHOD

Animals

Male Sprague-Dawley rats were purchased from Zivic-Miller for these experiments. They were housed individually for 1 week

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¹ Requests for reprints should be addressed to G. Jean Kant.

in an animal housing area with controlled lighting (12 h light: 12 h dark) and with food and water freely available prior to being placed in the operant cages. Rats weighed approximately 350 g at the beginning of each experiment.

Food

Food was supplied as 45-mg pellets. The sweeter pellet was purchased from Bio Serve Inc. (Frenchtown, NJ). The Bio Serve Dustless Precision 45-mg pellets (#0021) consisted of approximately 63% carbohydrate (53% sucrose), 18% protein, and 5% fat, and supplied 3.8 kcal/g. The other food (Noyes Precision Food Pellets, improved formula A) was purchased from PJ Noyes Company (Lancaster, NH). The Noyes Formula A 45-mg pellets consisted of approximately 60% carbohydrate (5% sucrose), 24% protein, and 4% fat, and supplied 3.7 kcal/g. The primary difference between the two food pellets was the sucrose content, although differences in the type of protein, fiber, etc., provided by each pellet may also have been present. Both companies supply a variety of pellets; the two types used were chosen from supplies on hand in the laboratory.

Experimental Procedures

Three preliminary studies and one preference study were performed. Each study utilized a different group of 12 rats. All rats were placed in standard operant cages housed inside a sound-attenuating chamber (cages, levers, chains, cue lights, chambers, etc., were purchased from Coulbourn Instruments). Houselights in the box were on from 0900 to 2100 h daily.

In the preliminary studies, one side wall of each cage had a centrally placed food trough to which pellets were delivered following a press on a single lever located on one side of the trough. The pellet dispenser contained either the high-sucrose or the low-sucrose pellet. The water bottle was placed on the other side of the food trough. In each preliminary study, six rats received the high-sucrose pellets and six received the low-sucrose pellets.

For the preference study, an additional lever was installed and the water bottle was moved such that each cage contained two levers, one located on each side of the food trough, which was centrally placed in one side wall immediately below the single water bottle. A second pellet dispenser was also connected and Y-shaped tubing carried pellets from either dispenser to the same food trough. Water was available ad lib. Pressing one of the levers delivered a high-sucrose pellet, while pressing the other lever once delivered the low-sucrose pellet.

All rats were allowed to habituate to the housing and levers until lever pressing for food pellets appeared stable. Four days were allowed for this period in the preliminary studies and 16 days in the preference study. Then, the experimenter trained four of the rats assigned to the controllable stress group (stressed) to pull a ceiling chain to escape scrambled foot shock delivered by the experimenter. The ceiling chain was suspended from the center of the cage and a 5-cm diameter metal ring was attached to the end of the chain, which hung approximately 10 cm off the floor. During this escape training, each controlling rat was yoked to a second rat (assigned to the uncontrollable stress or yoked group) such that the yoked rat received shock simultaneously with the rat being trained to escape shock. After each controlling rat was trained, a procedure that generally required no more than 15 min of intermittent shock, shock delivery was subsequently controlled by a PDP11 computer programmed in SKED (21). Shock presentation trials began with a 5-s illumination of a triple cue lamp, followed sequentially by a 5-s sonalert auditory warning tone, and then 5 s each of 0.16, 0.32, 0.65, 1.3, and finally 2.6 mA of foot shock. Trials could be avoided

or escaped at any point in the trial sequence by the controlling rat pulling the ceiling chain. The yoked rats had no control over shock delivery. In previous experiments, we have found that the rats with control over shock delivery avoid or escape more than 99% of the approximately 280 trials presented each day.

For the first 35 trials, shock trials were initiated at average intertrial intervals of 1 min. Following 35 successful escapes, the average intertrial interval was increased to 5 min. The computer was programmed to stop shock delivery if 20 consecutive escape failures from shock trials occurred; however, this condition was never met. Lever press and chain pull data were collected and stored by the PDP11 for daily analysis.

In the preliminary (one food choice) studies, lever pressing data were collected and analyzed for the 4 days prior to stress initiation and for the 7 to 14 days during stress. In the preference study, data were collected during the 16-day baseline period, during the 14 days of the sustained stress paradigm, and for an additional 7 days following stressor termination. One day of data during the poststress period was lost during a power failure. Finally, to assure that rats were actually demonstrating a food rather than a lever preference, the two types of pellets were switched so that each lever delivered the alternate food. This phase was continued for 5 days. Water bottles and animals were weighed daily.

RESULTS

Preliminary Studies

In order to determine whether significant differences in food intake would occur if the usual pellet used in our paradigm (the high-sucrose pellet) was changed to the low-sucrose pellet, we tested the two pellets in several experiments immediately preceding the preference experiment that is the main subject of this report. We filled half of the feeders for each group of rats (control, stressed, or yoked) with the high-sucrose pellet and the other feeders with the low-sucrose pellet. In each of three experiments, we collected data on two controls for each diet, on two stressed rats maintained on each diet, and on two yoked-stressed rats maintained on each diet, thus obtaining a group *n* of 6 for each condition and diet. As shown in Table 1, when only one food choice was available, rats lever pressed more for the low-sucrose pellets, especially in the control condition, than for the high-sucrose pellets (783 vs. 587). Stress, as expected, decreased lever pressing for both types of pellets during the first week of stress exposure. However, there was a significant interaction between stress and food type, such that there was a greater decrease in lever pressing in the stressed animals pressing for the low-sucrose pellets. However, this difference may be due to a smaller than usually seen decrease in the effects of stress on pressing for the

TABLE 1
LEVER PRESSES FOR HIGH- AND LOW-SUCROSE PELLETS
WHEN ONLY ONE TYPE OF PELLET IS AVAILABLE

Group	Low-Sucrose Pellets	High-Sucrose Pellets
Control	783 ± 15	587 ± 12
Stress	580 ± 33	473 ± 16
Yoked	533 ± 28	522 ± 24

Values represent the mean ± SEM for six animals in each group over the first 7 days of stress condition. Two-way ANOVA revealed significant effects of food pellet, $F(1, 190) = 31.7$; $p < 0.0001$, stress condition, $F(2, 190) = 32.5$; $p < 0.0001$, and their interaction, $F = 8.1$, $p < 0.0001$.

high-sucrose pellets. In many previous experiments [(e.g., (5,13)), we have seen decreases in responding for high-sucrose pellets equivalent to the decreases seen here for the low-sucrose pellets. This discrepancy may be simply due to the relatively small number of animals in each group.

Food Preference Prior to Stress

Rats rapidly learned to lever press for both types of food pellets, demonstrating individual variability in food preference. For example, on the last day of the baseline data collection, the average daily percentage of lever presses for sweeter pellets for all 12 rats was $71 \pm 4\%$ (422 pellets of 602). However, one rat ate 44% sweeter pellets (296 of 673), while another ate 94% sweeter pellets (524 of 558). The preference of individual rats appeared to remain fairly stable over time after the first few days, with the variation shown in the prestress period for rats 2 and 6 in Fig. 2 being representative.

Food choice was affected by time of day. As shown in Fig. 1, rats usually pressed very little for either food in the hours immediately following lights on, steadily increased pressing for the sweeter pellets during the 8 h preceding lights off without increasing pressing for the less sweet pellets, and then pressed in bursts of activity during the dark hours for both pellets, with more presses for the sweeter choice. Lever pressing peaked immediately after light offset. Reanalysis of the data collected in the preliminary experiments (above) revealed that when only one pellet type was available, the circadian patterning for either pellet resembled that seen for the high-sucrose pellet in the preference experiment, i.e., increasing lever pressing in the hours preceding lights off.

Rats were then assigned to control, stressed, and yoked groups on the basis of the 16-day baseline data collection so that prestress preferences could be roughly counterbalanced among groups.

Food Preference During Chronic Stress

Overall, there was no correlation between the stress period and food preference. One animal in the yoked group [see Fig. 2(A)] appeared to increase preference for the sweeter food choice during the stress period and to decrease the percentage of sweeter pellets delivered immediately following stressor termination. The pattern shown for another rat in the yoked group [Fig. 2(B)] was

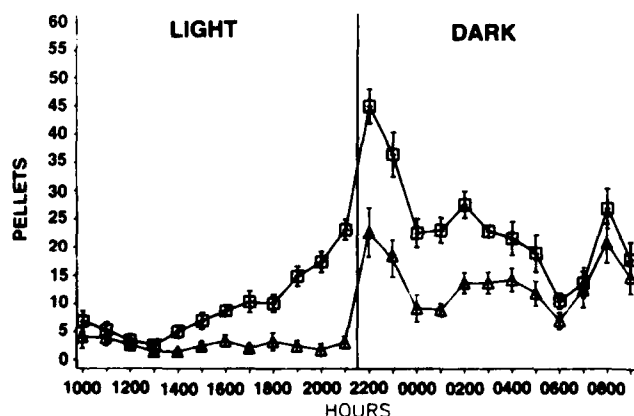


FIG. 1. Circadian pattern of lever pressing for a sweeter and less sweet food choice. Data are the mean from all 12 animals on the day prior to stress onset. Squares represent lever presses for the high-sucrose pellets and triangles lever presses for the low-sucrose pellets.

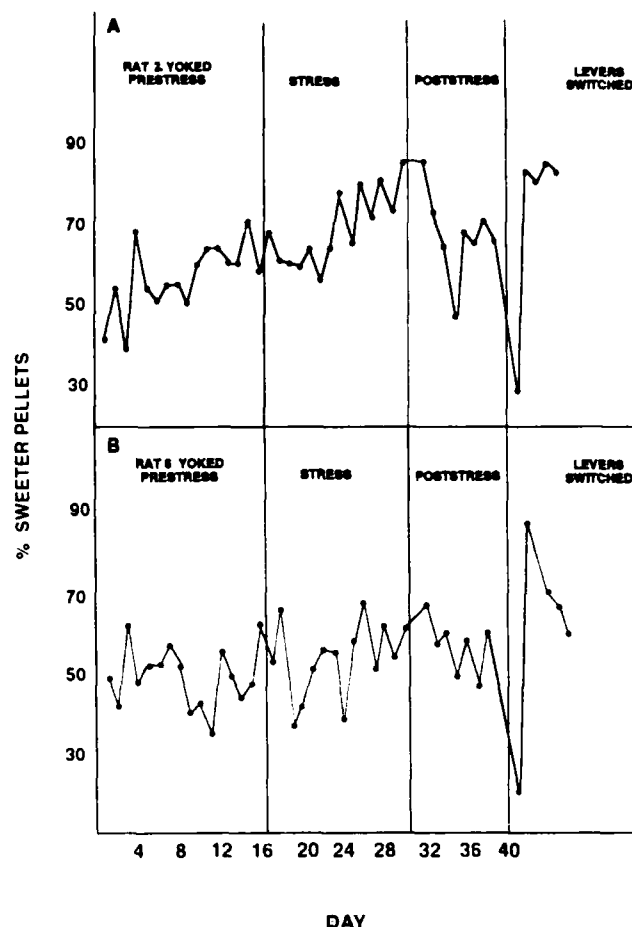


FIG. 2. (A) Effects of stress on a rat from the yoked group that increased preference for the sweeter food choice during stress. (B) Effects of stress on food choice in a rat in the yoked group that did not change food preference in response to stress.

typical of that seen for the other seven rats in the stressed and yoked groups, i.e., no marked changes during stress.

There were also no consistent changes in the circadian patterning of food preference as a function of stress, although individual rats on particular days did show disrupted eating patterns. Stress did decrease total lever presses for both food choices by approximately 30% on the first day of stress exposure, as expected from previous experiments. Lever pressing gradually returned to control levels over the first week of stress. Water intake was also decreased in both the stressed and yoked groups for the first day of the stress paradigm, but returned to prestress levels by the second day (data not shown).

When the levers were switched, following the end of the poststress data collection period, lever pressing quickly tracked to the new lever placements so that food preferences were maintained.

DISCUSSION

In previous studies from this laboratory utilizing a similar paradigm to the one described in this report, we have consistently found that the stressed and yoked-stressed animals decrease lever pressing for food for the first several days in the paradigm. Gen-

erally, the yoked animals take longer to return to prestress feeding levels, and plasma corticosterone levels are also higher in the yoked animals, suggesting that the yoked condition is more stressful (5,13). In these studies, only one type of pellet was available to the rats. The Bio Serve high-sucrose pellet used in the present study was the standard pellet used in our laboratory. More recently, the PJ Noyes formula A pellet with less sucrose has been the pellet used in our around-the-clock stress model. As reported herein, when only one type of pellet has been available, rats lever pressed at roughly similar rates with similar temporal patterning for either pellet.

The present study attempted to determine whether chronic stress would increase preference for the sweeter pellet when both choices were simultaneously available. Not surprisingly, the sweeter pellet was preferred even in the absence of imposed stress. Overall, in the present study, rats preferred the sweeter pellet approximately 2:1 over the less sweet alternative. Our results are quite similar to the data of Kanarek and Marks-Kaufman, and Castonguay et al. (8,12). In their studies, rats given access to both a standard laboratory diet and sucrose solutions chose to obtain approximately 40% to 60% of their total calories from consumption of the sucrose solution.

Preference was clearly affected by time of day. During the hours of darkness, when lever pressing rates were highest and rats earned most of their daily food, rats pressed for both types

of pellets proportionately, with the sweeter pellet preferred. Following lights on, almost no lever presses for either pellet were made during the first 4 h. Finally, during the last 8 h of light, lever pressing for the sweeter pellets increased steadily, while almost no presses for the less sweet pellet were recorded. It is tempting to speculate that this increased preference for sucrose at times of the day that do not correspond to major feedings is also seen in human snacking.

With regard to the major hypothesis of the present study, we did not find that stress increased preference for sucrose, with the possible exception of one animal. Although laboratory rats are bred to be more homogeneous than human populations, we observed fairly large variabilities in the preference for sucrose among animals, even in the prestress period. Thus, whether the one animal that increased its preference for the high-sucrose pellet represents a subset of animals that respond to stress by increasing sucrose intake (when available) or whether the data from this animal are simply anomalous cannot be determined without much more data. Further experiments to determine the incidence of stress-induced alterations in food preference in a larger sample of rats are necessary to resolve this question.

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